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CYTOGENETIC STUDIES OF MESENCHYMAL STEM CELLS IN RABBIT: A MINI-REVIEW

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ABSTRACT

The aim of the present review is to summarize current knowledges of *in vitro* studies, focused on the determination of rabbit stem cells of different origin, based on their cytogenetic examination. Stem cells represent valuable model to study the biological traits or processes of health and targeted tissues, affected by various internal or external detrimental factors. Furthermore, these cells provide a promising mechanism of treatment of existing human or animal diseases. Although recent knowledges based on serious *in vitro* studies bring positive promises, there are still remained a lot of issues focused to the safety of stem cell usage in the context of their clinical application. In this way, the stability of the genome across individual generations of passaged cells plays an important role, evaluated on the basis of chromosomal profile, including aneuploidy and structural studies. In the given context, various culture conditions and manipulations among the studies play a crucial role in the definition of the final chromosomal status. Up to date, there are numbers of reliable animal models used as donors of embryonic or somatic stem cells. In this way, the rabbit represents an available source with numerous advantages for cytogenetic analysis.

Key words: stem cell; chromosome; rabbit

INTRODUCTION

Stem cells are undifferentiated cells capable of self-renewal and of differentiation into specific terminally-differentiated cell types. Based on the source of derivation, they can be divided into somatic stem cells (SSCs) or embryonic stem cells (ESCs) (Rebuzzini *et al*., 2015). Somatic stem cells exert a crucial role in the maintenance of tissue homeostasis and participate in the repairing processes within their specific tissue. The ESCs are able to differentiate into almost all mature foetal and adult cell types, and thus they are defined as pluripotent cells (Cockburn and Rossant, 2010). While in the embryonic stem cells various chromosomal disorder has been widely reported, the mesenchymal stem cells are characterized as genetically stable during culture (Borgonovo *et al*., 2014). Mesenchymal stem cells (MSCs) are present in many adult tissues (Kang *et al*., 2012), capable of high proliferation and multi-lineage differentiation (Jin *et al*., 2013). Bone marrow was the first tissue, where the MSCs (BM-MSCs) were identified. Stem cells with this kind of origin possess various advantages as are: high osteogenic differentiation capacity, well investigated properties applied in use with biomaterials and not ethically controversial background (Till and McCullough, 1961; Kang *et al*., 2012). Invasivity of the BM-MSCs harvesting initiates the interest in finding more accessible sources of MSCs (Pontikoglou *et al*., 2011). SSCs have been identified in many different organs (i.e. skeletal muscle, heart, liver, fat, umbilical cord blood or placenta) (Rebuzzini *et al*.,

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2015). The potential use of stem cells (SCs) for tissue engineering (Katari *et al*., 2015), regenerative medicine (Grompe, 2012), disease modelling (Merkle and Eggan, 2013), toxicological studies (Seiler and Spielmann, 2011), drug delivery (Li *et al*., 2008) and as *in vitro* model for the study of basic developmental processes implies large-scale *in vitro* culture (Rebuzzini *et al*., 2015). *In vitro*, SSCs display greater plasticity, showing higher differentiation potential than *in vivo*. *In vivo*, SSCs can differentiate either in one, few or multiple cell lineages and, thus, are classified as unipotent (e.g. spermatogonia, oogonia), oligopotent (e.g. neural stem cells, NSCs) or multipotent (e.g. hematopoietic SCs) (Jiang *et al*., 2002; Franco-Lambert *et al*., 2009). The perspectives of stem cell clinical use is coupled with a serious issue about potential risk of forming tumours, the migration far away from the site of infusion and colonization of other tissues, the dedifferentiation of SC-derived differentiated cells, the establishment of an incorrect epigenetic and genetic status and an abnormal chromosome complement (Rebuzzini *et al*., 2015). On the basis of above mentioned facts, it is necessary to provide studies deeply focused on the determination of cytogenetic traits across generations of cultured stem cells. This advice is in accordance to several papers with evidence of stem cells difficulty to maintain a correct chromosome complement during prolonged expansion (Rebuzzini *et al*., 2011; Oliveira *et al*., 2014). As a promising animal model and the donor of stem cells for such studies, the rabbit has several advantages not only due to physiological manipulations – more easily carried out than those in mice, but also it is phylogenetically closer to primates than are rodents (Wang *et al*., 2007). For example, several authors have focused their force to study the rabbit embryonic stem cell behaviour under *in vitro* conditions (Fang *et al*., 2006; Wang *et al*., 2007).

Culture Conditions and Chromosomes

The effect of *in vitro* conditions on genomic stability of cells attracts the attention during the last years. However, the variability among culture protocols applied in different laboratories for derivation and culture of SCs complicates the identification of the source of such variations. The techniques used for cell detachment and disaggregation seem to be a major factor affecting the maintenance of genome integrity during long culture. Mechanical or manual methods (pipetting, flushing until the colonies are detached and disaggregated) are more gentle – less aggressive passaging techniques for subculturing and preserve better genome integrity than the use of enzymes (trypsin, collagenase) (Buzzard *et al*., 2004; Mitalipova *et al*., 2005; Lefort *et al*., 2008). To accelerate steps focused to disaggregation a modified enzymatic dissociation solution (0.25 % trypsin, 0.1 % collagenase IV, 20 % KSR, and 1 m M $CaCl_2$ in PBS), in combination with manual dissection for bulk passaging, has been proposed for hESC dissociation, demonstrating the maintenance of a normal chromosome complement even after more than 100 passages (Suemori *et al*., 2006). Several studies were focused on oxygen concentration during culture, but with contrasting results. Some studies suggested to use the O_2 concentrations between 1 to 7 % to significantly reduce the incidence of aneuploidies in hMSCs (Holzwarth *et al*., 2010; Li *et al*., 2011; Tsai *et al*., 2011; Estrada *et al*., 2012); whereas, the others recorded increased risk of aneuploidies and microsatellite instability in mouse NSCs, human bone marrow MSCs and human adipose SCs under the O_2 concentration between 1 and 5 % of chromosomes, even at early passages (Oliveira *et al*., 2012; Ueyama *et al*., 2012). High rates of aneuploidy, gaps and breaks were reported in hESC lines cultured under 21 % concentration of the O_2, in comparison with lower concentrations (Forsyth *et al*., 2006; Lim *et al*., 2011). A fundamental component of the SC medium is the serum. However, its animal (calf or bovine) or artificial (knockout serum only used for ESC culture) origin does not seem to play a role in the maintenance of genome stability (Inzunza *et al*., 2005; Ludwig *et al*., 2006). Similarly, the choice of a cell feeder layer (mouse embryonic or immortalized fibroblasts) or of supportive matrixes (gelatine, fibronectin, etc.) during the derivation and maintenance of stem cell lines does not seem to influence either the onset or the restraint of aberrations in stem cells genome (Cowan *et al*., 2004; Draper *et al*., 2004; Guo *et al*., 2005; Maitra *et al*., 2005; Mitalipova *et al*., 2005; Imreh *et al*., 2006; Sugawara *et al*., 2006; Rebuzzini *et al*., 2008).

Techniques for Karyotype Analyses

Several techniques are currently available to investigate the integrity of the chromosome complement of a cell line. Each method has advantages and disadvantages in terms of sensitivity, resolution and final costs (Catalina *et al*., 2007). Conventional banding techniques (G-, Q- or DAPI) allow a snapshot of the entire chromosome complement and the ordinary gross karyotype control of a cell line. These techniques, providing 300–400 stained bands, facilitate the detection of incorrect chromosome numbers (aneuploidies), mosaicism and large structural chromosome abnormalities, such as translocations, deletions or insertions.

Chromosomal status of cultured cells

Number of studies on chromosome variability have been performed on human mesenchymal stromal cells (MSCs) derived from the bone marrow. Independent laboratories reported contrasting results on the accumulation of chromosomal aberrations during *in vitro* culture (Ben-David *et al*., 2012; Sensebé *et al*., 2012). Some authors reported that human bone marrow-derived MSCs remain chromosomally stable throughout long-term culture, whereas others claimed the occurrence of numerical and structural chromosome aberrations within passages of *in vitro* culture (Pittenger and Martin, 2004; Soukup *et al*., 2006; Bernardo *et al*., 2007; Zhang *et al*., 2007; Sensebé *et al*., 2012). The study of Tomkova *et al*. (2017), focused on the aneuploidy determination of G-stained rabbit endothelial (peripheral blood) and mesenchymal (bone marrow, 3rd passage) metaphase stem cells, shows 73.3 % and 66.6 % diploidy or 26.6 % and 33.5 % aneuploidy, respectively. The results are partly similar to those of Kovacik *et al*. (2017), who focused to the chromosomal status monitoring of cultured rabbit stem cells isolated from the amniotic fluid and detected aneuploidy in the first three passages as follows: 18.18 %; 25.81 % and 23.53 %, respectively. Mentioned authors, on the basis of statistical analysis outputs, found no significant difference in the aneuploidy presence between stem cell cultures evaluated at various passages. These findings are in accordance to the results of Asadi-Yousefabad *et al*. (2015).

CONCLUSION

Current science provides wide options and promises in the use of stem cells with an expected success. This statement is supported by many scientific studies performed on a wide spectrum of stem cells isolated from and applied to different animal models. However, there are still remained studies with inconsistent results, which reveal not only positive, but also negative issues coupled with isolation, culture or determination of evaluated samples. Based on this fact, it seems reasonable to perform *in vitro* experimental studies in order to bring more detailed and clear answers, how to extract benefits from stem cell features in human and animal field. In this way, cytogenetic studies are useful tool to acquire early information about the genetic background of growing cells in the context of their future clinical use, basing on the chromosomal number and structure.

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